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Establishing *Quercus ilex* under Mediterranean dry conditions: sowing recalcitrant acorns versus planting seedlings at different depths and tube shelter light transmissions

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Abstract

Success of Mediterranean dry areas restoration with oaks is a challenging goal. Testing eco-techniques that mimic beneficial effects of natural structures and ameliorate stress contributes to positive solutions to overcoming establishment barriers. We ran a factorial experiment in a dry area, testing two levels of solid wall transmission of tube shelters (60 and 80%) plus a control mesh, and two depths (shallow and 15 cm depth) of placing either planted seedlings or acorns of *Quercus ilex*. Microclimate of the planting or sowing spots was characterized by measuring photosynthetically active radiation, temperature and relative humidity. Plant response was evaluated in terms of survival, phenology, acorn emergence and photochemical efficiency (measured through chlorophyll fluorescence). We hypothesize that tube shelters and deep planting improve *Q. ilex* post-planting and sowing performance because of the combined effects of reducing excessive radiation and improving access to moist soil horizons. Results show that temperature and PAR was reduced, and relative humidity increased, in deep spots. Midsummer photochemical efficiency indicates highest level of stress for oaks in 80% light transmission shelter. Optimum acorn emergence in spring was registered within solid wall tree shelters, and maximum summer survival of germinants and of planted seedlings occurred when acorns or seedlings were placed at 15 cm depth irrespectively of light transmission of shelter. Survival of germinants was similar to that of planted seedlings. The importance of techniques to keep high levels of viability after sowing recalcitrant seeds in the field is emphasized in the study.

Keywords Holm oak; Planting depth; Tree shelters; Chlorophyll fluorescence; Forest restoration; Direct seeding

Introduction

Restoring Mediterranean forests is frequently a challenging task. Summer drought is a major cause of failure (Villar-Salvador et al. 2012), and inter-annual variability in rainfall distribution is particularly high in the driest extremes of the precipitation gradient (Cortina et al. 2013). Other abiotic and biotic factors affect survival and growth of young seedlings. Excess of radiation and high temperatures in summer reinforce the stressing effects of drought (Gómez-Aparicio et al. 2008; Niinemets and Keenan 2014). Competing vegetation can limit the access to soil water (Cuesta et al. 2010). Predation by small mammals, birds or ungulates constitutes a major source of failure in some Mediterranean ecosystems (Pulido and Díaz 2005; Leverkus et al. 2013). Besides, under certain levels of degradation (croplands with intensive farming, surface mining or highly eroded soils) systems lose most of the structural elements and the sources of microsites diversity become limited (Kribeche et al. 2012). In addition, many restoration projects require plant densities and spatial distribution that do not match current structural layout of the biological legacy or microtopography. Under these circumstances, ecotechnologies at reasonable cost that mimic microsite amelioration effects and reduce predation have to be applied to ensure plant establishment.

One of the most widespread cultural practices in restoration programs in the Mediterranean over the past 20 years is the use of tube shelters (Oliet et al. 2003). Along with protecting seedlings from animal predation, a positive effect of shelter on survival has been observed in controlled studies (Piñeiro et al. 2013). Many studies show that these shelters improve survival of shade tolerant Mediterranean species (Oliet et al. 2003; Padilla et al. 2011). These results suggest a positive effect of tube shelters due to light reduction (Puértolas et al. 2010) and support the rationale of searching for an optimum wall transmission that minimizes negative impacts of excessive radiation without depleting root development (Jiménez et al. 2005). Vázquez de Castro et al. (2014) conducted a tree shelter light transmission study with Mediterranean species concluding that minimum light transmission of 40% should be considered as a target when designing shelters that promote biomass allocation to roots and improve water balance of Mediterranean seedlings. A more specific adjustment of light transmission to functional traits must be obtained by testing, under field conditions, a gradient of wall shelter transmission interacting with other stress factors during establishment, such as high temperature and radiation.

Other techniques like deep planting can be used to improve survival in dry areas. Deeper soil horizons have higher soil moisture during the Mediterranean summer (Padilla and Pugnaire 2007). Therefore, deep planting provides a more direct access to water and fosters root development within hydrated soils. However, deep planting can leave part of the active shoot covered with soil, which cause reductions in photosynthetically active foliar tissues and planting failures (Domínguez-Lerena et al. 2001; Hainds 2004). Deep planting using solid wall tube shelters can counteract this effect by preventing shoot from being covered by soil. A recent study using this technique shows improvements in survival of planted seedlings in a very harsh-dry Mediterranean area (Oliet et al. 2012). To our knowledge, this is the only published study with this technique, and additional characterization of microsite conditions around deep planted seedlings is needed, in particular when different light transmission of tree shelters are combined. This information will be useful to design the most appropriate combination to match niche regeneration requirements of introduced species.

Seed sowing or seedling planting are universal methods for establishing woody plant species. In the Mediterranean, both methods have been employed for restoration, although planting is the most popular (Pausas et al. 2004; Cortina et al. 2011). Planting has several advantages over sowing, such as a faster shoot growth and reduced mortality from seed predation, but is a more expensive method as it needs more investment in nursery producing seedlings and planting effort (Löf et al. 2004; Dey et al. 2008; González-Rodríguez et al. 2011). There is an intense debate about the most appropriate method for effectively guarantee survival [see for example Castro et al. (2015, this issue) and references therein]. No consensus exists, as the response to reforestation method is highly context-dependent. For Mediterranean forest restoration, heavy summer drought can reduce the options of sown seeds to germinate, emerge and grow roots in depth. This can be particularly important in species such as oaks, whose recalcitrant acorns lose viability very easily when humidity drops bellow high values (Villar-Salvador et al. 2013). In addition, large seeds of Mediterranean oaks attract predators due to the amount of energy available (Castro et al. 2006). However, some studies show better survival results sowing Mediterranean oaks even under dry conditions (McCreary and Tecklin 2001; Navarro et al. 2006), providing seed quality and predation is under control. The use of tree shelters associated to sowing operation of oaks is far from new in the Mediterranean (Carreras et al. 1996; Oñoro et al. 2001), and have rendered good results (McCreary and Tecklin 2001) probably due to combined effects of acorn protection and microclimate amelioration. Additionally, solid walled tree shelters allow placing the seed in deeper, wetter soil layers as compared to ground level without burying the acorn in excess, which could reduce plant performance (Seiwa et al. 2002). The use of shelters for deep sowing could be an innovative way of improving results of direct seeding with Mediterranean oaks.

The aim of this experiment is to analyze the combined effects of tube shelter light transmission and soil depth placement in establishment of planted seedlings and germinants of *Quercus. ilex* L. subsp. *ballota* (Desf.) Samp. Treatments are aimed to combine levels of soil water availability (planting/sowing depth) with irradiation (light transmission) during establishment, which are main drivers of establishment success in the Mediterranean (Gómez-Aparicio et al. 2008). *Q. ilex* is a slow growth, evergreen sclerophyllous oak that dominates many forest communities in the Mediterranean basin (Villar-Salvador et al. 2013). It is valuable species for restoration of woodlands in western European and northern African Mediterranean regions. However, seedlings of *Q. ilex* have high mortality and slow growth compared to other Mediterranean species (Oliet et al. 2003; Villar-Salvador et al. 2013). Natural regeneration success of *Q. ilex* is particularly sensitive to changes in irradiance (Puerta-Piñero et al. 2007), especially in dry sites (Gómez-Aparicio et al. 2008). Planting experiment was undertaken under stressing conditions of the limits of this species distribution (Villar-Salvador et al. 2013), to reliably test the effectiveness of these techniques. We are not aware of previous similar studies looking simultaneously at depth and light transmission of shelters in a planting-sowing experiment. We hypothesised that reduced light levels and deep planting in the shelter enhance performance of both transplanted seedlings and direct sown germinants. The information derived from this study can provide rationale about how to improve the establishment of this species in restoration projects, with potential beneficial implications in the restoration of other similar water limited ecosystems.

Materials and Methods

Study site

The study site is located in a recently abandoned flat cropland in central Spain (La Mancha, 39º22' N, 3º14' W, 640 m asl, Alcázar de San Juan) which had been cultivated for grain. The climate is Mediterranean continental, with mean annual precipitation of 417 mm and a mean annual temperature of 15.2 ºC. Summers are very hot and dry, and last for 3-5 months, and winters are cold with frequent frosts (Ninyerola et al. 2005). Soils are deep and have alluvial origin (Instituto Geológico y Minero 1991). Four randomly chosen soil samples were taken at 0-30 depth cm to characterize pH (8.5±0.1) and texture (11.6+0.8% sand; 51.4+0.5% silt and 37.0+0.6% clay, silty-clay-loam USDA category). Accumulated rainfall from January to September (averaged data from 1992 to 2012) is 246.4 mm, while precipitation during the same months in the planting year (2012) was lower than average (187.2 mm): hence, May 2012 was particularly dry, as only 18.9 mm was registered compared to 57.5 mm average from 1992-2012; rainfall in June and July was negligible until September, when precipitation was higher than average (data from meteorological station of National Agency of Meteorology).

Origin and production of reproductive material

Acorns of *Q. ilex* were collected from ES12 “La Mancha” provenance region (Alía-Miranda et al. 2009) during fall 2010 and stored to reduce viability losses following Villar-Salvador et al. (2013) method. Prior to sowing in the nursery or in the field, a floating selection procedure was followed to discard damaged acorns (González-Rodríguez et al. 2011). The selected seed-lot had an average dry weight of 2.4±0.091 g (n=30) per acorn after removing the pericarp from the cotyledons. For the planting experiment, the seedlings were produced in the School of Forestry campus (Technological University of Madrid, 40°27′N, 3°43′W, 664 m asl). One seed per container (230 cm^3 volume, 16.5 cm length, Super Leach, Bardi, Navarra, Spain) was sown in March 2011 at a growing density of 308 $seedlings \cdot m^{-2}$. Seedlings were raised in peat moss substrate fertilized with Osmocote Plus 15-11-13+2 Mg (Scotts Co., Marysville, OH, USA) at a 4 $g \cdot l^{-1}$ rate. Prior to planting, seedlings height and root collar diameter were 12.5±0.2 cm and 3.6±0.0 mm, respectively (n=200), which meet European regulatory requirements for planted *Q. ilex* seedlings (Directive 1999/105/EC). For the direct sowing experiment, the same seed-lot as per planting was used to avoid confounding effects due to genetic differences and year of collection. One month prior to field seeding, a subsample of 100 acorns was removed from storage to determine seed viability by placing the acorns on four trays filled with vermiculite in a growing chamber at 20ºC. Total number of fully germinated acorns was counted after four weeks as a measure of viability (ISTA 2011). Viability of the seed-lot prior to direct sowing in the field was 75%.

Field experiment

The site was subsoiled in October 2011 at a 60 cm depth with rippers 50 cm apart to improve soil properties of this former farmland. Seedlings or acorns were placed in manually opened holes (0.3 m × 0.3 m × 0.3 m) at 3.0 × 3.0 m spacing. The experimental design was a factorial experiment, and treatments were a combination of the following factors levels: 1) forestation method (direct seedling versus planting); 2) tube shelter type (solid wall light transmission of 60 and 80% and mesh); and 3) depth of planting or sowing (0 and 15 cm depth, shallow and deep, respectively).

For direct seeding treatments, three acorns per sowing point were sown in a circle limited by the tube and introduced at a depth of 3-5 cm in the manually opened hole to ensure emergence (González-Rodríguez et al. 2011). Planting or sowing depth treatments consisted of placing the top of the plug or the acorn at ground level (shallow, but considering the 3-5 cm to keep the acorn covered) or 15 cm below ground level (deep). For deep planted seedlings or sown acorns, the bottom of the shelter was introduced 15 cm under the ground level to prevent the shoot from being covered with soil (Oliet et al. 2012).

The solid wall tube shelters tested were made from plastic material supplied by Repsol Química (Spain). Additives were added to the copolymer base to reach the light transmissivities tested in this experiment, maintaining the red/far red ratio around 1 (neutral shade) (Vazquez de Castro et al. 2014). Hand-made tubes using the plastic sheets were circular, single-walled tubes, 50 cm tall×10 cm wide, with four ventilation holes facing each other of 2.5 cm width and situated at 18 and 36 cm in height. In addition, a mesh protection with shallow planted or sown *Q. ilex* was used as a control. Mesh tree shelter consisted of a 60 cm tall×15 cm wide cylindrical blue polyethylene net with mesh holes 0.8×0.8 cm (Redplanton, Projar SA, Valencia, Spain) and a light transmission coefficient of 83%; mesh size is big enough to allow normal air circulation so no differences exist in air temperature and relative humidity between the inside and outside of the shelter (Vazquez de Castro et al. 2014).

Treatments were arranged in experimental units consisting of rows of ten plants or sowing points each. Four experimental units per treatment were randomly assigned (n=40 plants or sowing points per treatment), and a total of 200 seedlings and 200 sowing points (600 acorns in total). Planting and sowing were conducted in January 2012. Pre-emergence herbicide (4 g oxifluorfen$\cdot l^{-1}$) was sprayed to the soil after planting around 50 cm of each planting or sowing spot. Manual weeding around the seedlings was conducted in May, and continuous mechanical disc trenching at the end of June.

Evaluation of microclimatic conditions

To assess the effect of light transmission of tube shelters and depth on microclimatic conditions around seedlings, air temperature and relative humidity (RH) data loggers sensors (Hobo U23-001® Onset, USA) were installed in the shelters from May 12 to September 5 2012. Three Hobo probes were randomly installed inside shelters of each treatment with a living seedling. For shallow planted and mesh, hobo sensors were set at a height between both pairs of ventilation holes (27 cm), while probes for deep planted treatment were placed at 7.5 cm above ground level inside the shelter. Photosynthetically active radiation (PAR) was measured with quantum sensors (SQ-212®, Apogee, USA) connected to Hobo U12 data loggers. Sensors were previously calibrated. One PAR sensor 2 cm tall was used per treatment and placed inside a shelter without a plant. Thus, for deep spots, PAR sensor was placed at 15 cm bellow the general field ground level, and for shallow planted seedling the sensor was placed at 0 cm. Sensors were leveled horizontally to reduce experimental sources of error due to differences in radiation incidence angles. Two periods of 4-5 sunny days were used in winter (19 to 23 January) and summer (28 June to 2 July) to characterize radiation availability across the treatments tested. Every sensor of the experiment was programmed to record current data at a 15 minutes interval.

Monitoring plant response to treatments

To characterize the response of the plant material to treatments, phenology of the planted seedlings, acorns germination, and survival dynamics of both seedlings and germinants were monitored from planting throughout spring and summer. To characterize developmental stage of planted seedlings, a scale of five phenological stages was used. Stages (0—terminal bud formed; 1—swollen terminal bud; 2—

growing leaves; 3—leaves formed but not fully expanded; 4—leaves fully expanded) were observed only on the main stem. When a second growing flush occurred, upper correlative numbers were used for subsequent phenological stages. Phenology monitoring was concluded when no significant changes were observed between two consecutive censuses.

Acorn emergence was assessed at one week interval during the spring and first days of summer. Emergence was recorded when leaves of germinants were visible. Therefore, emergence includes the germination of the acorn plus the growth of the epicotyl up to the surface. Acorn emergence is presented as the number of germinants relative to acorns sown. After maximum percentage of acorn emergence was registered (around June 15th), alive seedlings emerged was assessed on a monthly basis till September 06. Survival of planted seedlings was recorded as changes in mortality occurred from June first to September 06. Rainfall events after this date concluded the dry season. At this time, additional performance-variables were calculated to characterize sowing response to treatments: survival of germinants, calculated as the proportion of plants remaining alive relative to maximum number of acorns emerged, and plot success as the proportion of sowing points with at least one plant established relative to total sowing points (González-Rodríguez et al. 2011). Survival of germinants can be used to statistically analyze both forestation methods in conjunction.

To provide a physiological basis for the plant response to the experimental conditions applied, we measured photochemical efficiency by chlorophyll fluorescence during midsummer. Chlorophyll fluorescence measurements were conducted on six randomly chosen plants per treatment on two consecutive sunny days in July (25 and 26, three plants per treatment per day). The measurements were made in a fully expanded leaf of the upper third of the seedling through a window opened in the shelter wall. The ratio of variable to maximum fluorescence (F_v/F_m) was measured at noon with a pulse-modulated fluorometer (FMS®, Hansathech Instruments, UK) as a surrogate of photochemical efficiency. Prior to F_v/F_m measurements, leaves were dark acclimated for 30 min (Kalahi et al. 2014).

Data processing and statistical analysis

Temperature and RH data were averaged from each 15 minutes interval and mean daily values per sensor were calculated. Daytime values of temperature and RH were determined by excluding the period between sunset and dawn. Temperature and RH data were analyzed over the measurement period using repeated-measures one way analysis of variance (RM-ANOVA) with shelter (nested within depth, see below) as a between-subject factor, and day as the within-subject factor. Statistical differences among temperatures and RH were identified using Fisher's protected least significant difference (LSD) test, adjusting the overall α level by Bonferroni correction. Accumulated PAR radiation per day was calculated by integrating each 15 minute interval, and mean daily value from each period is given for winter and summer periods. For data analysis of phenology and survival of the planted seedlings, as well as percentage of acorns emerged, plot success, survival of germinants, and chlorophyll fluorescence of both seedlings and germinants, an ANOVA derived from a general linear model was conducted. In this ANOVA, as deep planting/sowing cannot be conducted with mesh, shelter type factor was nested within depth of planting or sowing. For mentioned variables except fluorescence, ANOVA was run with shelter type and planting depth as fixed factors. For chlorophyll fluorescence and for comparison of survival of germinants and planted seedlings, a three-way ANOVA was conducted that included also the forestation method fixed factor. Percentages were arcsine transformed prior to analysis, though data were reported as original means with standard errors. Effects were considered significant when P <0.05. All the analyses were carried out with the SPSS v.15.0 statistical package (SPSS Inc., Chicago, IL, U.S.A.).

Results

Microclimatic conditions

A significant effect of treatments were found for mean daytime temperature and RH through the summer ($F_{4,7}$ = 19.234 and $F_{4,7}$ = 20.972, respectively, P = 0.001 for both variables). Maximum mean diurnal temperature occurred within shallowly placed 80% light transmission tubes, being 4.1ºC higher than external air (Table 1). Temperatures in depth were almost the same irrespectively of light transmission, and did not significantly differ from those under external conditions. Shallowly placed shelters of 60% light transmission were 1.8ºC colder than 80% along the summer (Table 1); these differences were marginally significant (P = 0.092, Bonferroni test). Planting depth had a significant effect on RH, with mean values within deep shelters 9.4% higher than that for shallow shelters (Table 1). RH in latter treatments did not significantly differ from external air RH (Table 1).

During the winter period considered, mean accumulated PAR in a day did not strongly differ between solid wall transmission for a given planting depth. Differences between 80% and 60% light transmissions were 1.9 and 0.1 mol·m^{-2}·day^{-1} within shallow and deep planted spots, respectively (Table 1). At summer solstice, those differences raised to 16.4 and 7.1 mol·m^{-2}·day^{-1}. Conversely, relative differences in accumulated PAR during a day due to planting depth were much higher in winter (shallowly placed seedlings receiving in average 7.1 more PAR) than in summer (shallowly placed seedlings receiving in average 3.3 times more PAR, Table 1). During a mean day of the winter period studied, maximum PAR in depth was 90.6 (±3.1) $\mu mol \cdot m^{-2} \cdot s^{-1}$ within a deep 60% light transmission tube, with 5.4 h above 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (estimated light compensation point for *Q. ilex* seedlings growth at full sun, Gómez-Aparicio et al. 2006).

Phenological stage and survival of planted seedlings

A significant effect of shelter type on phenology was found from the first weeks of spring to the end of May (Fig. 1a). At the beginning of April, seedlings shallowly planted in clearest tubes (80% light transmission) showed the fastest phenological development, while those planted in mesh was the slowest. Despite no significant differences were found in phenological stage since June (when values started to peak), seedlings in clearest tubes and planted shallowly reached values of mean phenological stage clearly over 4 (4.9±0.6, Fig. 1a), indicating that some surviving seedlings (26%) started a second flush, although only 6% of surviving seedlings completed this developmental stage. Rest of treatments stabilized around 4, with only some exceptional seedling starting a second flush (data not shown). No significant differences in phenology of planted seedlings were observed by depth of planting during the period (*P* values increasing from 0.195 in April to 0.987 in July).

Survival of planted seedlings was significantly affected by planting depth. Seedlings started to show significant differences in mid June, when survival of shallow planted seedlings dropped drastically (Fig. 1b). Seedlings planted at depth had almost 100% survival till July, with values as high as 67.5±12.5% and 72.5±7.5% for 60% and 80% light transmission levels, respectively, by the end of this month. Among shallow planted seedlings, mortality of seedlings in mesh was slightly higher along the summer, but those differences vanished at the beginning of September, when mean survival rates of shallowly planted seedlings across shelter types dropped to 7.5±3.0%. By this time, survival of deep planted seedlings (18.8±4.4% in average across both light transmissions) was still significantly higher. For deep planted seedlings, there were no significant differences in survival by light transmission of the shelter wall (Fig. 1b).

Performance of sown acorns

As per planted seedlings, acorn emergence was mostly affected by depth of sowing, with acorns sown in depth having superior performance (Fig. 1c). During germination and emergence period (May and June) shelter type factor had also a marginal significant effect ($F_{1,15}$ = 4.5, P = 0.051 on June 04; $F_{1,15}$ = 2.5, P = 0.096 on June 15 and $F_{1,15}$ = 2.8, P = 0.075 on July 04). On June 15, when the maximum proportion of living germinants across treatments occurred, acorn emergence in mesh was the lowest (28.3±8.3%), while emergence in solid wall shelters was maximum (averaged value for all treatments in solid wall shelters 53.1±3.2%). As summer progressed, the percentage of emerged to total sown acorns at depth remained significantly higher than that from acorns sown shallowly irrespective shelter type. At the end of the studied period (September 06) this percentage for deep sown germinants was 19.6±4.5% in average across 60 and 80% light transmissions, while germinants from acorns sown in shallow dropped to almost no survivors (3.0±1.3% across all types of shelters, Fig. 1c). In addition, final plot success for deep sown acorns averaged across light transmissions of the solid walls shelters (36.2±7.3%) was also significantly higher ($F_{1,15}$ = 18.5, P = 0.001) than that for shallowly sown seeds (7.5±2.8%). Survival of germinants in September (relative to maximum number of emerged plants) was also significantly affected by sowing depth ($F_{1,15}$ = 14.8, P = 0.002). Acorns germinated in depth had higher survival (34.5±7.8%) than acorns that emerged at shallow spots (8.2±2.8%). Both plot success and survival of germinants in September was not affected by shelter type factor (ANOVA not shown).

Chlorophyll fluorescence of planted seedlings and germinants

Values of maximum photochemical efficiency (F_v/F_m) were between 0.1-0.5. Both planting/sowing depth ($F_{1,50}$= 7.1, P = 0.010) and shelter type ($F_{1,50}$ = 5.8, P = 0.020) significantly affected this variable (Fig. 2). Chlorophyll fluorescence in the plants growing in 80% tubes was lower, but only in the shallowly placed shelter. No differences between planted and sown seedlings were found ($F_{1,50}$ = 0.8, P = 0.382). In addition, germinated acorns and planted seedlings followed the same patter in relation to shelter type and planting depth ($F_{2,50}$ = 1.5, P = 0.239 and $F_{1,50}$ = 0.3, P = 0.563, respectively).

Comparing planted seedlings and germinants

Survival at the end of the studied period was not significantly affected by forestation method ($F_{1,31}$ = 2.3, P = 0.144), with average values across treatments of 12.0±2.8 and 18.7±4.5% for planted seedlings and sown germinants, respectively. Forestation method did not interact neither with depth of planting or sowing nor with tree shelter type (data not shown). Plot success of the sown points was in average 19.0±4.6%.

Discussion.

Acorn emergence and survival of seedlings and germinants. Effect of planting depth

Deep planting or sowing improves environmental conditions for plants during the summer, resulting in increased RH and reduced daytime temperature and radiation. Additionally, light conditions in depth do not limit vegetative activity, as daily PAR values in winter at basal stem (darkest spot) height (-15 cm) in the 60% type shelter were various hours over light compensation point for *Q. ilex*. Similar values of PAR were found in other experiments with holm oak (Leiva et al. 2013), with no depletion of germination and survival.

Our study demonstrate that the first phase of germination and emergence of acorns is favored by sowing acorns in depth, but also marginally by shelter type, with lower values for acorns in mesh. Time lapse between sowing and emergence was five months, in which recalcitrant acorns of *Q. ilex* can lose viability if not adequately preserved (Villar-Salvador et al. 2013). Higher soil and air humidity in deep spots helped to preserve viability. Acorn viability of this species is highly sensitive to depletion of humidity, but not to temperature changes over a relatively wide range (Zulueta and Montoto 1992). According to Smit et al. (2009), lower emergence of acorns sown in openings as compared to those placed under shrubs is due to a protecting effect from desiccation. However, we did not observe differences in air RH between mesh and solid-walled tree shelters in shallow spots (Table 1). Solid wall tube shelters can condensate water from dew, slightly improving soil water content of the upper cm (del Campo et el. 2006). In summer, when germinants mortality is the main constrain to sowing success, shelter type factor loses its marginal significance and depth of sowing becomes more significant. Deeply sown spots clearly showed higher percentage of living germinants, survival of germinants and plot success than shallow sown seeds irrespectively of shelter type.

Survival of planted seedlings mimics this response to treatments, with higher values for deep planted seedlings in every type of tree shelter. It has also been observed even under harsher conditions (Oliet et al. 2012). This study showed higher soil humidity in depth, with effects on water status of seedlings in midsummer. Mortality of seedlings follows soil moisture drying dynamic in the profile (Padilla and Pugnaire 2007), and this can explain the dramatic drop in survival since the beginning of the summer for shallow planted seedlings.

Under the heavy drought of the area and the planting year, significant improvements in survival for deep planted/sowed seedlings indicates that this technique can be appropriate for water limited ecosystems.

Planting versus sowing acorns

Comparison of both forestation methods across treatments showed no significant differences between survival of planted seedlings and that of germinants. Despite low survival rates at the end of the summer reduce conclusiveness of the results, our study indicates that direct seeding of oak could render similar results than planting in harsh conditions. Mortality values of planted seedlings and germinants after emergence is identical, and is not differently affected by treatments. Some authors have highlighted the differences in root growth pattern of planted seedlings and germinants in this species (Pemán and Gil, 2008; Tsakaldimi et al. 2009). Harsh conditions of our experiment (in particular the low rainfall rates in key months for survival as May and June) could have inhibited the performance differences in the field among both stocktypes. Plot success is a combination of emergence percentage and number of acorns sown. The first depends upon acorn viability prior to sowing and on microenvironmental conditions after sown in the field. Increasing the number of acorns per sowing point plot improves plot success, but acorn availability can be a limiting factor. Choosing direct seeding method for this oak and other recalcitrant species implies certainty of high seedlot viability right before sowing (Cole et al. 2010), but also creating the post-sowing conditions that preserve it prior to emergence.

Effect of tree shelter type

We did not find significant differences in plot success and survival of germinants or seedlings by tree shelter type, as per other studies of planted *Q. ilex* (Oliet et al. 2003; Navarro-Cerrillo et al. 2005). However, we did find significant differences in shoot phenology of planted seedlings by tree shelter type, with solid-wall tubes accelerating more advanced growing stages as compared to mesh. This effect is particularly clear for seedlings growing within the light and warm 80% tube probably because, providing other resources are not limiting, shoot growth initiation in the spring is controlled by air temperature (Abramoff and Finzi 2015). Bud bursting and vegetative activity earlier in spring can be an interesting advantage for planting in the Mediterranean, where summer drought can occur at the very beginning of the summer. Prior study with this species analyzing seedling growth under controlled conditions in shelters shows earlier root growth for sheltered *Q. ilex* in the spring (Puértolas et al. 2010).

Chlorophyll fluorescence as an indicator of stress level for each treatment

Photochemical efficiency measurements provide evidences of the combined effect of high irradiation, drought and temperature on vegetative status during the experiment. The observed values of maximum photochemical efficiency on July (Fig. 2) were well below the threshold (0.8) for healthy non-stressed plants (Björkman and Demmig 1987). This reveals that the surviving plants were subjected to very stressful conditions which triggered mechanisms of photoprotection or even provoked photodamage, both resulting in photoinhibition (Ball et al. 1995). Photoinhibition is expected to be more pronounced with increasing photosynthesis limitation and with increasing radiation levels (Demmig-Adams and Adams, 2006). Therefore, the lowest values of F_v/F_m recorded in seedlings growing in the shallow solid wall 80% light transmission tube, reveals that the combination of drought, higher temperatures and high radiation of this treatment (Table 1) induced the highest degree of photoinhibition. Previous study with planted *Q. ilex* under a wider gradient of tube wall transmission reports optimum values ($F_v/F_m > 0.8$) for all of them, probably because that experiment was conducted under no water deficit (Vázquez de Castro et al. 2014). Although high irradiation combined with drought is the main cause of seedlings mortality in this species (Gómez-Aparicio et al. 2008), no clear relationship between photoinhibition levels and survival was observed in our study. Particularly hard conditions of our experiment reduced survival of shallow planted/sowed seedlings to negligible values irrespectively of light transmission. This confirms that photoinhibition in July is mostly the consequence of photoprotective mechanisms and mortality is probably more strongly linked to other factors like access to water, as the differences between shallow and deep planted seedlings suggest. However, the impact of light levels on plant survival should not be neglected, as deep planting or sowing not only is likely to facilitate root access to deeper wetter soil layers, but also decreases radiation levels which could reduce plant transpiration and, in turn, the risk of embolism during extreme drought events. Disentangling the positive effect of these two combined factors associated to deep planting could be essential to design the most cost-effective technique for *Q. ilex* forestation in semiarid environments.

Conclusions

Under conditions of dry and hot summers, planting/sowing at depth renders better survival of *Q. ilex* than shallow planting/sowing. Deep planting or sowing spots provide milder environmental conditions to seedlings in summer, increasing air humidity and reducing temperature and radiation. This study also shows that sowing *Q. ilex* under semiarid conditions could be a viable alternative to planting in former cropland providing acorns are sowed in depth and assisted by solid-walled tube shelters. This treatment improves micro-ambient conditions for preserving viability, germination and emergence of *Q. ilex* acorns. Although the effects on survival are not conclusive, chlorophyll fluorescence results suggest maximum stress levels of *Q. ilex* growing inside 80% light transmission level. These conclusions are not only interesting for oak but for many other nut-recalcitrant producing tree species to be used in restoration of dry ecosystems. But further experimentation testing the effects of planting/sowing depth at longer term under different environmental conditions will provide more conclusive results on the benefits of this technique.

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Table 1

Table 1. Microclimatic characterization inside two types of solid wall light transmission shelters (60 and 80%) placed at two depths (0 and 15 cm). Mean temperature and RH are mean diurnal values (±SE) from May 12 to September 5, 2012. Values of temperature and RH followed by the same letters do not differ at 0.05 significance level (Bonferroni post-hoc test). Photosynthetically active radiation (PAR) is the mean (±SE) of accumulated radiation each day of two periods in winter (January) and summer (June-July).

	Exterior	Shallow-80%	Shallow-60%	Deep-80%	Deep-60%
Summer daytime temperature (ºC)	32.2±0.4c	36.3+0.4a	34.5+0.3ab	32.9+0.4bc	33.0+0.3bc
Summer daytime RH (%)	25.8+1.2b	22.9+1.2b	26.1+1.0b	33.4+1.2a	34.4+1.0a
PAR radiation (mol·m^{2}·day^{-1})					
- Winter	NA	12.0+0.5	10.1+0.7	1.6+0.0	1.5+0.0
- Summer	61.8+0.7	44.6+1.6	28.2+0.3	14.7+0.2	7.6+0.1

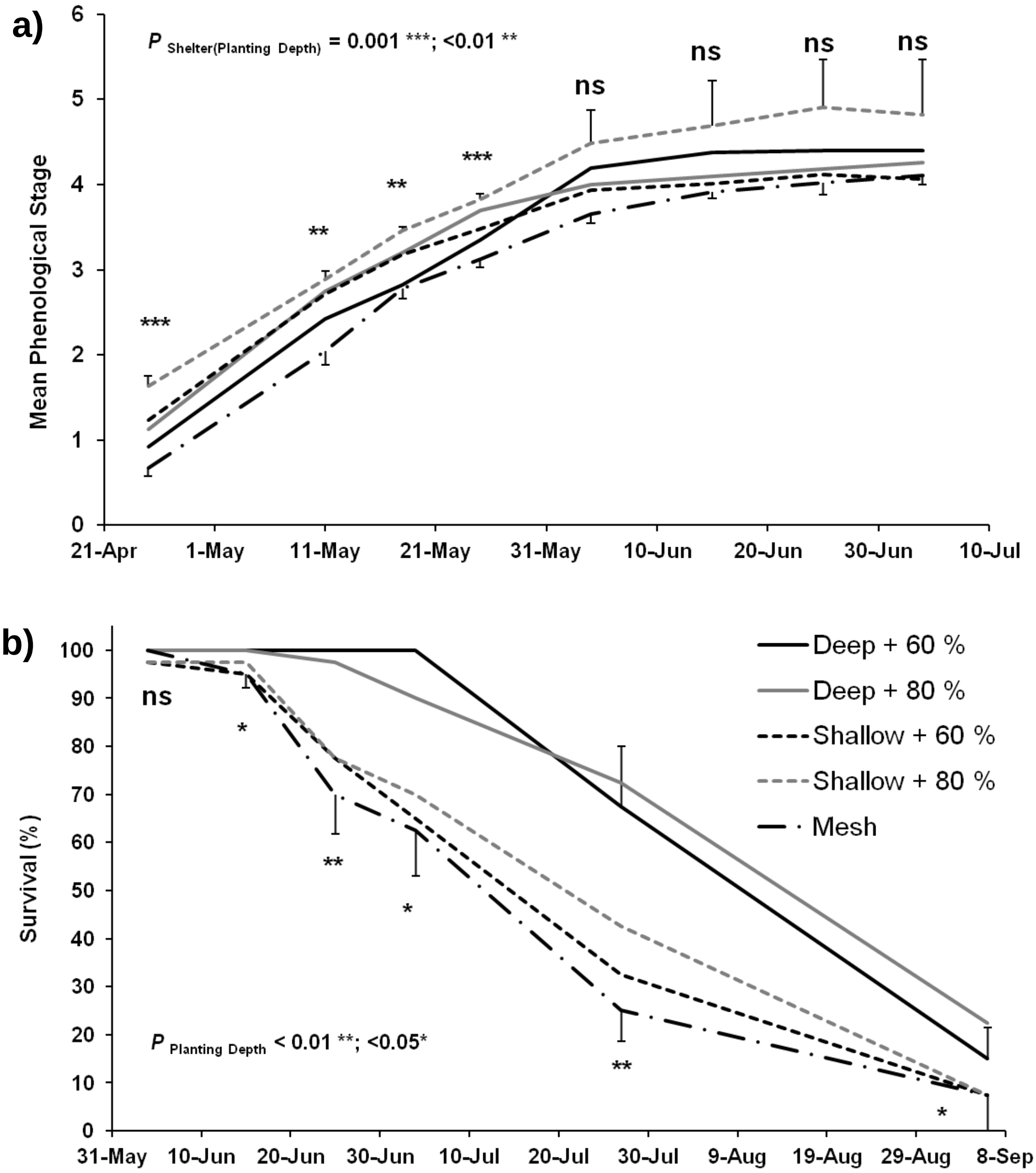
NA External radiation during winter period could not be recorded

Figure captions

Fig. 1 Phenological stage (a), survival percentage of planted seedlings (b) and percentage of acorn emergence (c) of *Q. ilex* in a planting and sowing experiment as affected by tube shelter type and planting/sowing depth. For each response variable, *P* values of the significant factor from the ANOVAs conducted along the period are presented. For clarity, only SE of maximum and minimum treatments are shown (n = 4)

Fig. 2 Photochemical efficiency (F_v/F_m±SE) of germinants and planted seedlings of *Q. ilex* as affected by shelter type and planting/sowing depth (n = 6). *P* value of the significant main factors from the ANOVA are presented

Figure 1


a)
P Shelter(Planting Depth) = 0.001 ***; <0.01 **
Mean Phenological Stage
*** ** ** *** ns ns ns ns
0 1 2 3 4 5 6
21-Apr 1-May 11-May 21-May 31-May 10-Jun 20-Jun 30-Jun 10-Jul
b)
Survival (%)
ns * ** * ** *
P Planting Depth < 0.01 **; <0.05*
0 10 20 30 40 50 60 70 80 90 100
31-May 10-Jun 20-Jun 30-Jun 10-Jul 20-Jul 30-Jul 9-Aug 19-Aug 29-Aug 8-Sep
Deep + 60 %
Deep + 80 %
Shallow + 60 %
Shallow + 80 %
Mesh

c)

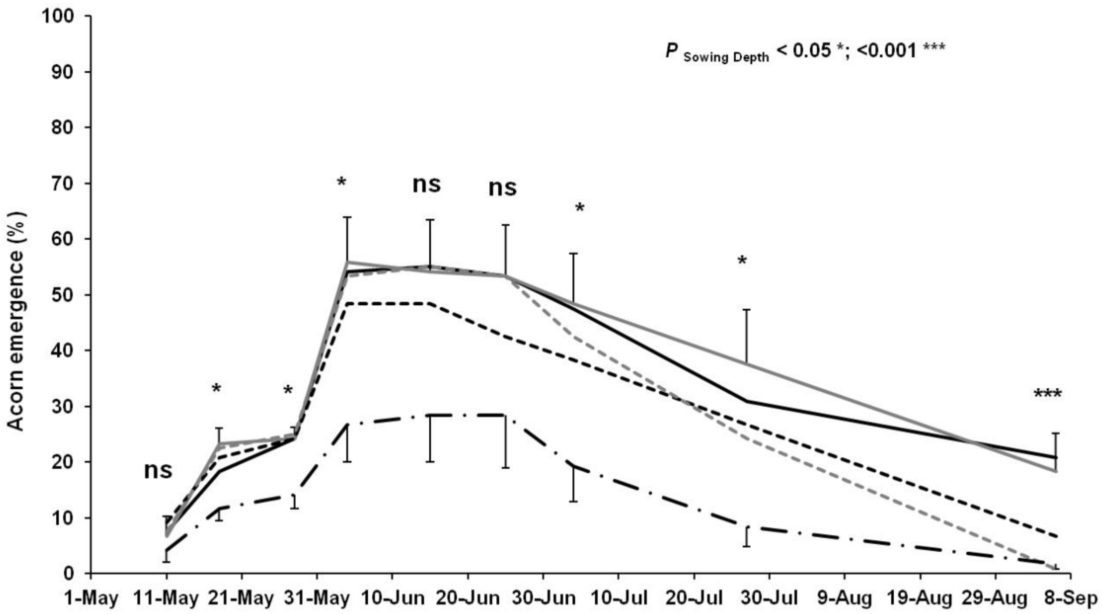
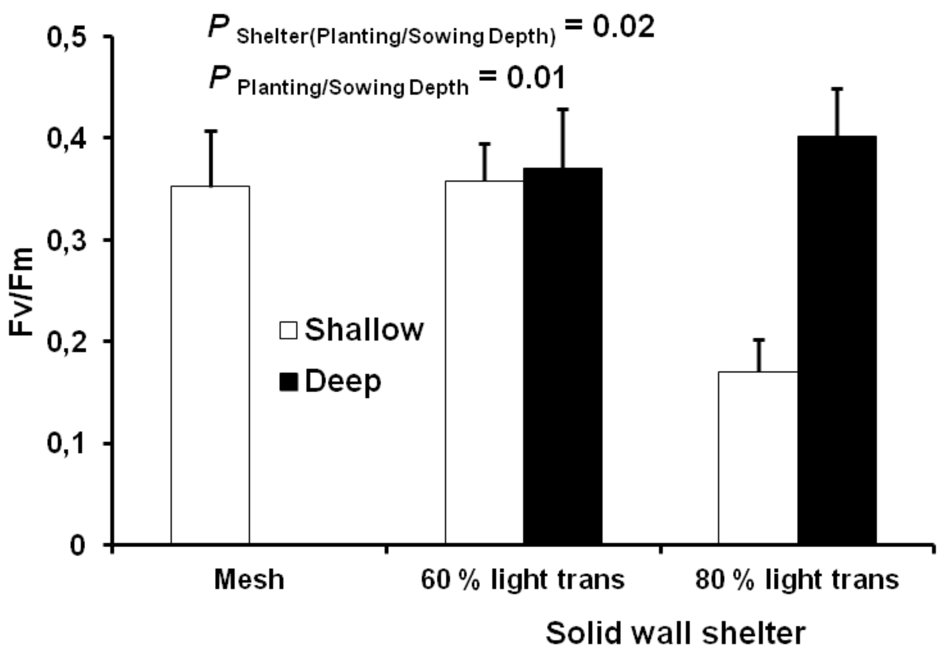

P Sowing Depth < 0.05 *; <0.001 ***
Acorn emergence (%)
100
90
80
70
60
50
40
30
20
10
0
ns
*
*
*
ns
ns
*
*
***
1-May
11-May
21-May
31-May
10-Jun
20-Jun
30-Jun
10-Jul
20-Jul
30-Jul
9-Aug
19-Aug
29-Aug
8-Sep

Figure 2


P Shelter(Planting/Sowing Depth) = 0.02
P Planting/Sowing Depth = 0.01
0,5
0,4
0,3
0,2
0,1
0
Fv/Fm
Shallow
Deep
Mesh
60 % light trans
80 % light trans
Solid wall shelter